Irradiation as an alternative phytosanitary treatment for Arhopalus ferus and Hylurgus ligniperda

Andre van Haandel[1] (Supervisors: Dr. Stephen Pawson[2], Dr. Tara Murray[1], and Dr. Sophie Walker[3])

1. School of Forestry, University of Canterbury, 2. Scion, Christchurch P.O Box 29-237, 3. ESR Christchurch P.O Box 29-181

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I. Abstract

Wood products all require treatment to mitigate phytosanitary risk prior to exportation. The most common phytosanitary treatment applied to *Pinus radiata* logs is Methyl Bromide (MeBr). The Environmental Protection Agency (EPA) in 2010 stated that MeBr must not be release into the atmosphere past 2020. This poses a problem for New Zealand log exports. Radiation has been identified as a possible alternative phytosanitary treatment for export wood products.

This study aimed to quantify the effective dose of radiation necessary to sterilise two forest pest species; *Arhopalus ferus* and *Hylurgus ligniperda*. These species are representative of two different types of forestry pests; bark beetles (*H. ligniperda*) and wood borers (*A. ferus*). All applicable life stages for both species were tested.

Arhopalus ferus adults were the most susceptible life stage identified with an LD99 of 30.2Gy ± 13.5 Gy (95% confidence interval). *Arhopalus ferus* eggs were less susceptible with a LD99 of 750Gy ± 776Gy observed; however there is low confidence in this result due to a methodological issue in one treatment replicate. *Hylurgus ligniperda* eggs were observed to be less susceptible than *A. ferus* eggs with a LD99 of 289Gy ± 92Gy. Results for the other life stages were inconclusive due to poor control survival, however the information gained was used to develop improved methods for further experimentation, which is on-going and showing positive results so far.

The results of this experiment have indicated that radiation can be an effective method of sterilising forestry pests. To date radiation has not been used as phytosanitary risk mitigation for wood exports; however it is widely used for risk mitigation in agricultural products. Currently there remains a large amount of unknown information regarding, the effectiveness for irradiation of logs, the effective dose require for sterilisation of the most tolerant forestry pest and public acceptability of irradiation as a phytosanitary treatment. These knowledge gaps and an economic assessment must be completed before irradiation can be used as a phytosanitary risk mitigation technique for forestry products.

II. Keywords

Irradiation, *Arhopalus ferus*, *Hylurgus ligniperda*, Forestry, Exports, Methyl bromide, Phytosanitary treatments, Sterilisation, Quarantine and Pre-Shipment (QPS).

III. Glossary

EDN	Ethane dinitrile
EPA	Environmental Protection Agency
ERMA	Environmental Risk Management Agency
FAO	Food and Agriculture Organization of the United Nations
GLM	Generalised Linear Model
GDP	Gross Domestic Product
Gy	Grays (unit of radiation)
ICPR	Importing Country's Phytosanitary Requirements
IDIDAS	International Database on Insect Disinfestation & Sterilization
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
LD	Lethal Dose
LD99	Lethal dose at which 99% of all treated individuals are sterile
MeBr	Methyl Bromide
MfE	Ministry for the Environment
MPI	Ministry for Primary Industries
NPPO	National Plant Protection Organisation
QPS	Quarantine and Pre-Shipment
SPS	Sanitary and PhytoSanitary Measures Agreement
WTO	World Trade Organisation

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Table of Contents

1. Introduction

International trade in wood products carries an associated biosecurity risk, as commodities can harbour plant pests and diseases. All export trade in wood products requires treatment to mitigate such phytosanitary risks. The most common form of treatment for export logs from New Zealand is Methyl Bromide (MeBr). The New Zealand Environmental Protection Agency (EPA) in 2010 stated, New Zealand must not release any MeBr into the atmosphere by 2020. This is due to MeBr being an ozone depleting substance with unknown effects on human health.

New Zealand's forestry sector accounts for 10.1% of New Zealand export earnings in 2012. In the last 10 years the amount of MeBr used in New Zealand has risen with the increasing volume of logs exported. New Zealand, like many countries, is heavily reliant on MeBr and must actively develop alternative phytosanitary treatments for logs to meet the EPA 2020 requirement.

A number of alternative phytosanitary treatments have been proposed, including ecologically based risk assessments, alternative chemical fumigants, and non-chemical approaches, such as heat and radiation. Radiation is used as a phytosanitary risk mitigation technique for a wide range of agricultural and horticultural products and could potentially be applied in a forestry context. Radiation can be used to disinfest (death) or sterilise pests and does so without affecting the quality of the export product. This makes radiation an attractive alternative for phytosanitary risk mitigation.

This study investigated and attempted to quantify the effective dose of radiation required to induce sterility in key forest pest species. Such results will inform a future cost benefit analysis to determine the cost-effectiveness of radiation as an alternative treatment for New Zealand forestry exports.

2. Literature review

2.1 Biosecurity risks

The New Zealand economy is reliant on primary industries, such as agriculture, horticulture and plantation forestry, which jointly account for 6% of Gross Domestic Product (GDP) and >50% of total export earnings (The Treasury, 2013). Trade in roundwood and wood products carries particularly high biosecurity risk of transporting quarantine pests from the country of export to the importing country. This is because forests pest species have the potential to colonise recently deadwood and can be transported undetected to an importing country.

A pest species, by definition, must pose a threat to New Zealand's economy, its environment, or in some cases human health (The Biosecurity Council, 2003). If a serious new forest pest were to establish, it may affect the health or productivity of forests resulting in a loss to GDP (Aukema et al., 2011). In response to these threats, countries may legally specify phytosanitary treatments to mitigate risks posed by imported commodities (World Trade Organisation (WTO), 1995). Countries are also focusing closely on 'export' biosecurity risks within their own primary production systems, not just those that affect production but risks that may limit their future export potential (TBC, 2003). The New Zealand forest industry requires robust procedures to minimise biosecurity risk to retain strong trade relationships.

2.2 International requirements for phytosanitary treatments

The World Trade Organisation (WTO) Sanitary and PhytoSanitary Measures Agreement (SPS) provides a framework for negotiating and formalising trade agreements between countries to minimise biosecurity risks, and provide a dispute resolution process (WTO, 1995). The SPS agreement specifies that phytosanitary treatments i.e. treatments applied to destroy pests or diseases associated with plant products, can only be imposed in response to recognised phytosanitary risks that have been identified as part of a pest risk assessment (WTO, 1995). In addition treatments imposed must be scientifically justified, to the minimum necessary to mitigate known risks, and that they are applied consistently and not arbitrarily (WTO, 1995).

Consistency refers to the requirements imposed by one country on different importing countries; if the pest complex is the same for a particular commodity then constraints cannot be arbitrarily applied beyond the minimum justifiable standard for one particular country relative to another (WTO, 1995).

The International Plant Protection Convention (IPPC) is the standard setting body for the WTO-SPS as it is applied to plants and plant products. The purpose of the IPPC is “To prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control” (Article 1.1) (International Plant Protection Convention, 1997). The IPPC uses the principles of the SPS agreement to develop standards, guidelines and recommendations to mitigate plant health risks. These standards are referred to as the International Standards for Phytosanitary Measures (ISPMs) and are followed by National Plant Protection Organisations (NPPO) when setting the Importing Country’s Phytosanitary Requirements (ICPR) for the trade in plant products (IPPC, 1997). The ICPR allows each country to set their own sanitary and phytosanitary requirements within the scope of the WTO-SPS conditions and requirements (IPPC, 1997).

2.3 IPPC recommendation on MeBr use and approval of alternatives

At present New Zealand is heavily reliant on Methyl Bromide (MeBr) for phytosanitary risk mitigation of insect pests in wood exports. As log export volume has increased, so has the amount of MeBr used for quarantine and pre-shipment (QPS) fumigation (Fig. 1) (Ministry for the Envronment (MfE), 2012). In 2012 New Zealand used 571.3 tonnes of MeBr for QPS purposes (MfE, 2012); this reliance on MeBr will remain until a range of approved alternatives have been developed to support trade. Although MeBr is permitted under the Montreal Protocol for QPS use, the IPPC recommended in 2008 that contracting parties put in place strategies to reduce the use, or emission, of MeBr for QPS use (IPPC, 2008). As an IPPC signatory New Zealand should actively seek alternative phytosanitary treatments to reduce its reliance on MeBr. The Environmental Risk Management Authority (ERMA) (now the New Zealand Environmental Protection Agency (EPA))

ruled in 2010, as part of a reassessment of the use of MeBr that New Zealand must not release any MeBr into the atmosphere by 2020 (Environmental Risk Management Authority, 2010).

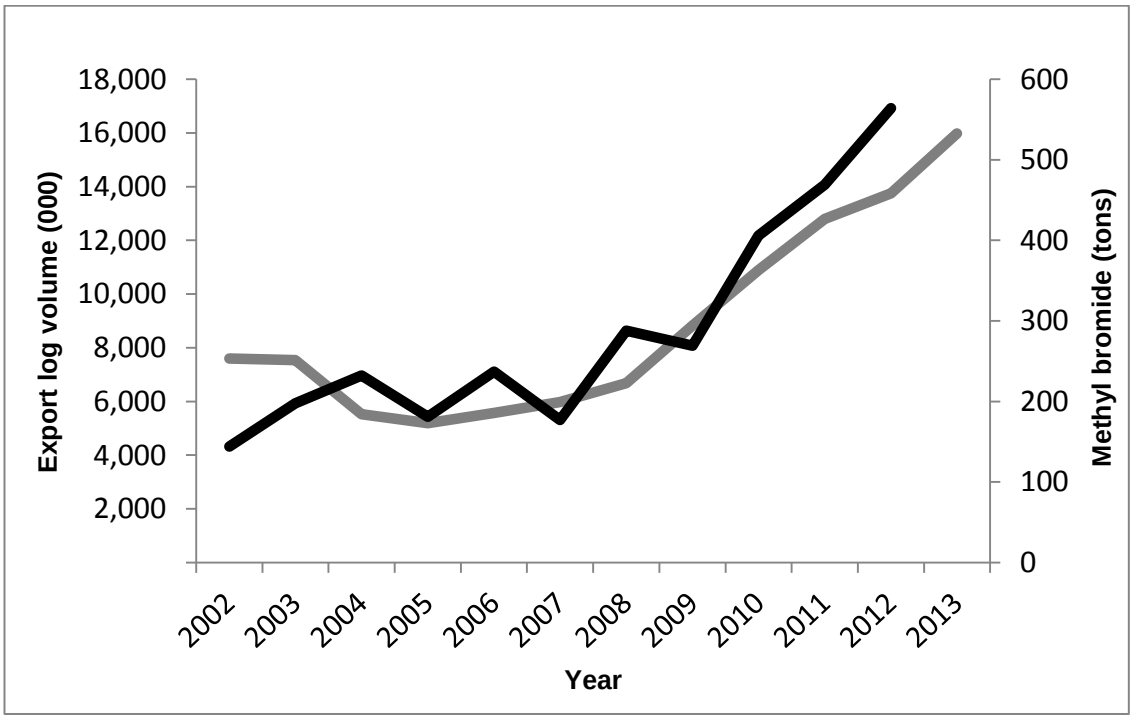

Figure 1. The increase in log exports (grey line) and MeBr (black line) in New Zealand over the past 11 years.

2.4 Alternative phytosanitary treatments

There are a limited number of potential alternatives to MeBr that could be applied to New Zealand forest products and these can be placed into three different categories;

1. Ecologically based risk assessment approaches
2. Alternative chemical approaches
3. Non-chemical approaches (including irradiation)

Ecological risk based approaches examines pest biology, to identify periods of time (such as during winter) or spatial areas, where pest pressure is sufficiently low that it negates the need for phytosanitary treatments. From a trade perspective these are referred to as pest free areas of production (IPPC, 2007). Also if the justification is a temporal, as opposed to a spatial, pest free area, then this approach would need to be combined with some form of treatment for the time of the year when pest insects are active.

Alternative chemical treatments to replace MeBr that have been identified are limited, but potentially include sulphuryl fluoride or ethane dinitrile (EDN) (Armstrong, Brash, & Waddell, 2014). Non-chemical (physical) treatments include various technologies such as heat, cold, irradiation, microwaves, or the removal of bark (Armstrong et al., 2014). These alternative treatments are not currently at an operationally feasible stage; however they are potential future options that require additional research. Phosphine will not fully replace MeBr as its use is restricted to in-hold cargo on ships and cannot be used for on wharf fumigations of top stow cargo, as it requires a 10 day treatment (Armstrong et al., 2014).

2.5 Radiation as a potential phytosanitary treatment for wood products

Radiation has been identified as a potential Phytosanitary treatment for wood products (Scion, 2012). Ionising radiation is radiation that carries enough energy to liberate electrons from atoms or molecules (Serway & Jewett, 2008). Gamma rays and X-rays are forms of ionizing radiation (Serway & Jewett, 2008). Grays (Gy), is a derived unit of ionizing radiation that is a measure of the absorbed dose, with 1 Gray defined as the absorption of one joule of radiation energy by one kilogram of matter (Taylor & Thompson, 2008).

Radiation is widely used as a phytosanitary treatment for agricultural and horticultural commodities. Two different thresholds are often applied for irradiation treatments, A) sterilisation or B) disinfestation. Sterilisation is defined as the minimum dose required to render an organism non-viable (Bakri, Mehta, & Lance, 2005), whereas disinfestation is defined as the minimum dose required to kill all pests (Ignatowicz & Zaedee, 1994). Sterilisation requires a lower dose of radiation (Bakri et al., 2005) and it is therefore more appropriate to look at sterility from a phytosanitary treatment perspective, than to look at mortality.

There are three main sources of radiation that can be used for phytosanitary applications. These are radioactive isotopes, electron beams (E-beam) and

X-rays. For the purpose of this dissertation a cobalt-60 source was used, because it was the only source available. Although Cobalt 60 is unlikely to be the source of choice in any future application of forest product irradiation in New Zealand, it is proof that the principal of ionising radiation can be used for such purposes.

2.6 Challenges with finding alternative phytosanitary treatments

A significant challenge when creating a new phytosanitary treatment is that they need to be scientifically justified, i.e., it must be proven that the treatments are effective at controlling the pest of interest. This process is time consuming and costly. Scientific justification must be based on the criteria given in ISPM 28 that provides objective measures of treatment efficacy that includes the number of replicates and individuals needed for experiments (IPPC, 2009). In addition ISPM 18 provides specific guidance for irradiation as a phytosanitary treatment and suggests a two phase test: A) determine the most resistant life stage of the species of interest, and B) conduct a confirmatory test on the most resistant life stage (IPPC, 2003).

Although it is not explicitly required, a testing protocol called Probit 9 has historically been required by standard setting bodies as proof of efficacy for new phytosanitary treatments (Haack, Uzunovic, Hoover, & Cook, 2011). First developed for fruit flies, Probit 9 requires the testing of at least 93,613 insects without any survivors. Probit 9 provides a high degree of certainty; however it is not practical to apply when evaluating forestry pests that can be difficult to breed. In response, Haack et al (2011) recommend an alternative 3-step process: 1) laboratory experiments to estimate the lethal dose for the most tolerant stage of each target pest, 2) replicated experiments (with no survivors) at the estimated lethal dose using a minimum sample size of 60 experimental units (0.95 statistical reliability at the 95% confidence level), 3) confirmatory tests using operational conditions and infestation levels similar to field conditions (Haack et al., 2011). This three step process is similar to ISPM 18, which was used in this study.

2.7 Current use of irradiation as a phytosanitary treatment

Irradiation has been used against many different insect species in multiple orders including Mango seed weevil, (Coleoptera); Fruit flies, (Diptera); Maskell beetle, (Hemiptera); Codling moth, (L) (Lepidoptera). Irradiation for phytosanitary risk mitigation has been used in agriculture for some time. For example, mangos exported from Hawaii to the US mainland are irradiated as there is no other approved quarantine treatment for the Mango seed weevil, *Cryptorhynchus mangiferae* (Follet, 2001). Adult weevils that emerged from samples treated with 100Gy and 300Gy treatments were lethargic and short lived and laid no eggs, indicating sterility (Follet, 2001). For the Maskell beetle, *Planococcus minor* 250Gy was found to sterilise or prevent generation turnover (Ravuiwasa, Lu, Shen, & Hwang, 2009). Codling moth, *Cydia Pomonella* is also irradiated as a phytosanitary treatment and at 200Gy adult emergence is prevented (Mansour, 2001).

An example of an agricultural pest is on tomatoes and capsicums which present an import risk to New Zealand, as they are a known host material for the Queensland Fruit Fly (*Bactrocera tryoni*), which is a listed quarantine pest (Ministry for Primary Industries, 2013). These products were previously treated with dimethoate however, concerns about human health risks associated with this treatment have resulted in a switch to irradiation as a means to manage their phytosanitary risk (Ministry for Primary Industries, 2013). The minimum absorbed dose of 400Gy is considered to be effective to prevent the introduction and spread of tomato and capsicum regulated pests (Ministry for Primary Industries, 2013).

Research has also been conducted on wood pests. In Taiwan, a Hemipteran called *Planococcus minor,* which is a horticultural pest feeding on the phloem of live trees (Ravuiwasa et al., 2009), has been identified as significant risk of establishing due to geography and environmental conditions. *Planococcus minor* has been identified as a significant pests to over 250 tree species in Afrotropical, Australasian, Nearctic, Neotropical and oriental regions (Ravuiwasa et al., 2009). It causes reduced yield, reduced fruit quantity, decolouration, indirect damage from sooty mould and can vector plant

viruses (Ravuiwasa et al., 2009). It was found for *P.minor* that 250Gy was enough to sterilise or prevent generational turnover (Ravuiwasa et al., 2009).

The only dose response treatment of a New Zealand wood export and forest pest, focused on the larval life stage of the Huhu beetle (*Prionoplus reticularis*) (Lester, Rogers, Petry, Connolly, & Roberts, 2000). Lester et al (2000) examined the dose required for disinfestation and found that very high doses (LD_{99}=3677Gy) were required. Further work is required to confirm the dose rates necessary to sterilise or disinfest export wood pests with sufficient confidence to define an acceptable phytosanitary treatment (Lester et al., 2000).

In summary, there has been significant research into the use of irradiation for phytosanitary risk mitigation for agricultural products, however little research has been conducted on forestry pests. For New Zealand to secure on-going export opportunities with our key trading partners, New Zealand needs to identify effective irradiation levels for our forestry pests. Supporting data must satisfy our major trading partners, such as China, that they are effective against specific forest pests. China's ICPR currently states that logs should be free of the following New Zealand forest pests *Hylurgus ligniperda, Platypus spp* and *Sirex noctilio* (Ministry for Primary Industries, 2013). This study is a first step towards determining whether irradiation is a feasible and justifiable alternative to using MeBr by testing two key phytosanitary pests of importance to China (*H. ligniperda*) and Australia (*A. ferus*).

2.8 Problem statement and research aims

New policies restricting the release of MeBr into the atmosphere come into force in New Zealand in 2020. MeBr fumigation is cheap, easy and is accepted by most countries as a phytosanitary treatment for export logs. Finding acceptable and sustainable alternatives to MeBr as phytosanitary risk mitigation for log exportation should be a priority for New Zealand, as currently New Zealand is heavily reliant on the use of MeBr, although there is the use of phosphine and debarking in certain situations. Irradiation has been identified as an alternative phytosanitary treatment for forestry pests on wood

products. The specific aim of this project is to quantify the minimum required dose of radiation necessary to induce sterilisation of the most tolerant life stage of *A. ferus* and of *H. ligniperda.* This dose will underpin a second phase of testing that will present a technical justification for the use of radiation as an alternative phytosanitary treatment to replace MeBr. Identifying this minimum effective dose also facilitates a techno-economic assessment of radiation to determine its operational viability as a replacement.

2.9 Hypothesises

Null hypothesis: There is no association between dose and sterility of applicable life stages of *A. ferus* and *H. ligniperda*.

Alternative hypothesis: There is an association between dose and sterility of applicable life stages of *A. ferus* and *H. ligniperda*.

3. Methods

3.1 Insect sources

Adults

Hylurgus ligniperda and some *A. ferus* were collected using ethanol and alpha-pinene baited panel traps from Ashley forest, Bottle Lake forest and West Melton forest (Fig 2a). The majority of *A. ferus* were collected at night by hand at the SRS Rolleston sawmill, with some also caught in the forest panel traps.

Eggs

Arhopalus ferus were paired for mating and eggs gathered from females left in containers at ambient room temperature following the method of van Epenhuijsen et al (2012). Eggs of *H. ligniperda* were collected using an artificial bark habitat that comprised two thin 12 cm×7 cm pieces of bark placed phloem-side down on a moist cloth inside a plastic vented 750 ml container (Fig. 2b). Twenty adult *H. ligniperda* were added to each sandwich and eggs extracted after 12 to 14 days.

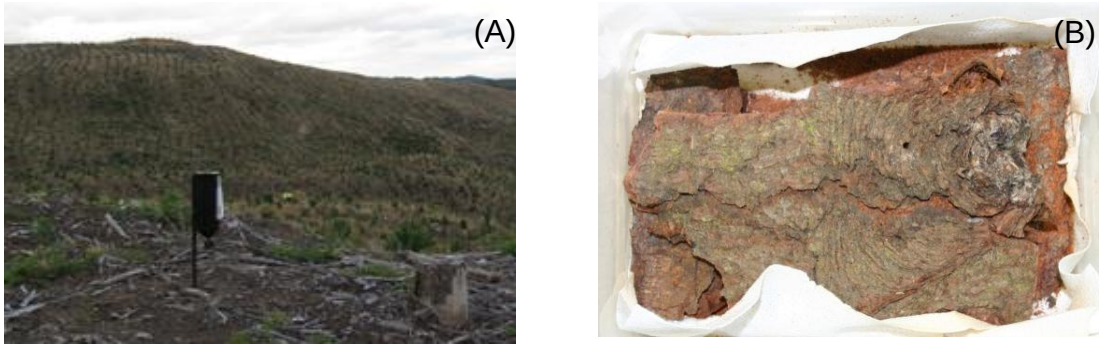

Figure 2. (A) A live capture field trap with attractants for the collection of *A. ferus* and *H. ligniperda* adults. (B) An artificial bark habitat used to collect *H. ligniperda* eggs laid by field collected adults.

Larvae and pupae

Larvae of both species were obtained by allowing some of the eggs to hatch. Neonate larvae were transferred to a Petri-dish of artificial diet. Where necessary these larvae were supplemented from field collected larvae by prising bark from logs, but field collected larvae were only used as a last resort (to replace individuals lost due to a controlled-temperature room malfunction). A proportion of the *H. ligniperda* larvae were also reared through to pupae.

3.2 Irradiation

Radiation was applied using a Theraton 80 that uses a cobalt 60 source to emit gamma radiation. Different radiation doses were delivered using the principle of the inverse square law, which states the intensity of the radiation is inversely proportional to the square of the distance from the source (Aparecida & Aquino, 2012). An adjustable stand (Fig. 3.) was used to place insects at the correct calculated distances away from the source, so that a range of doses could be applied simultaneously.

Figure 3. Theratron 80 machine and irradiation stand, which allows different doses to be applied to different dishes of insects simultaneously.

3.3 Pre-irradiation care

All life stages were stored in a temperature controlled chamber at a constant 10°C ± 1.5.Field collected *H. ligniperda* adults from the live capture traps were stored in Petri dishes in groups of 20 (Fig. 4a). For each experiment *A. ferus* individuals were stored individually in 10 ml plastic vials sealed with cotton wool (Fig. 4b).

For the second experiment, 20 *H. ligniperda* eggs were kept in impressions on top of artificial diet in quarter segment of a Petri dish (Fig. 5b). Clusters of 20 *A. ferus* eggs were cut from paper towels and placed on moist filter paper in a quarter section of the divided Petri (Fig. 5b). Adult *H. ligniperda* were placed in the remaining half segment of the Petri dish (Fig. 5b). Twenty *A. ferus* larvae were placed in two Petri dishes (ten in each dish) filled with artificial diet by cutting out a small wedge, inserting a larva, and then putting

the diet wedge lightly on top (Fig. 5a). Twenty *H. ligniperda* pupae and larvae were each put in a Petri of artificial diet (Fig. 5a).

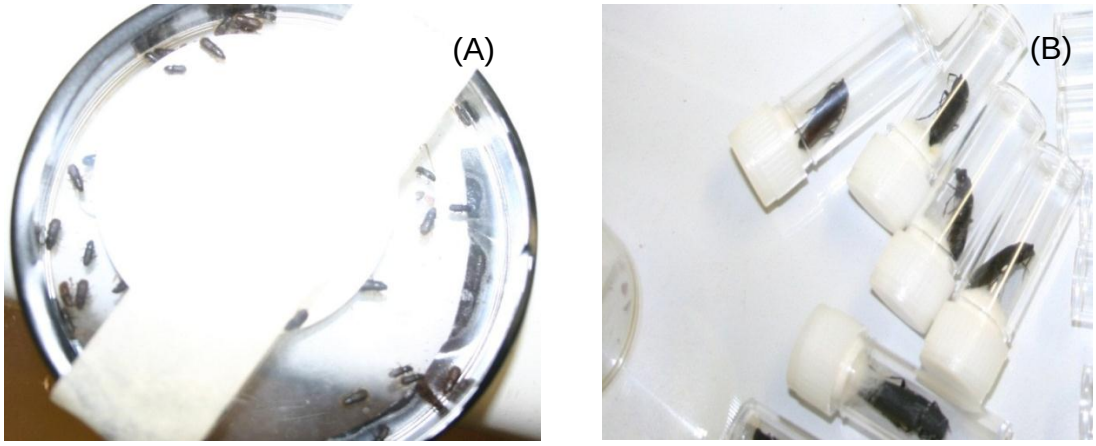

Figure 4. (A) Free-roaming *H. ligniperda* adults set up Petri dish immediately prior to the Experiment 1 irradiation. (B) *Arhopalus ferus* adults in individual 10 ml vials with cotton wool, prior to Experiment 1 irradiation.

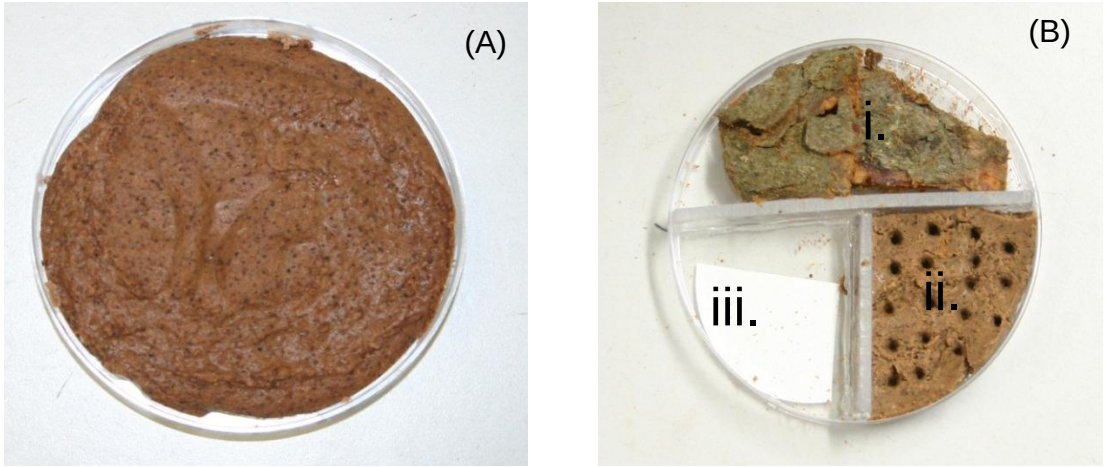

Figure 5. (A) Artificial diet in Petri dish onto which *H. ligniperda* larvae, pupae and *A. ferus* larvae were placed prior to Experiment 2. (B) (i) *Hylurgus ligniperda* adults on a piece of bark, (ii) eggs in impressions on artificial diet and (iii) *A. ferus* eggs on a piece of filter paper in a segmented Petri prior to Experiment 2.

3.4 Post-irradiation care

Post-irradiation both treated and control insects were maintained under favourable conditions in a temperature controlled chamber at a constant 20°C ± 1.5. Survival, development and reproduction were measured as these are the IPPC recommended parameters of sterilisation.

Hylurgus ligniperda adults were reintroduced into bark discs in a ventilated Petri dish (Fig. 6a). *Hylurgus ligniperda* pupae and larvae were left in the Petri's of diet (Fig. 6b). Once the pupae and larvae had reached adulthood they were moved onto a bark disc to monitor development (Fig. 6a).

The *A. ferus* adults were placed in opposite sex pairs in 100ml plastic containers with moistened paper towels and a square of bark (Fig. 7a). If *A. ferus* adults produced eggs during the irradiation they were left in the 10ml vials so as not to disturb or damage the eggs. Spare adults remaining after pairing were placed individually in vials. Each larva was transferred from the Petri dish of diet to an individual 10ml tube (Fig. 7b), after further development they were transferred to 100ml containers (Fig. 7c). *Arhopalus ferus* eggs were left in the segmented Petri on a moist piece of filter paper with a ventilated lid. *Hylurgus ligniperda* eggs were left in the segmented Petri on impressions on the diet.

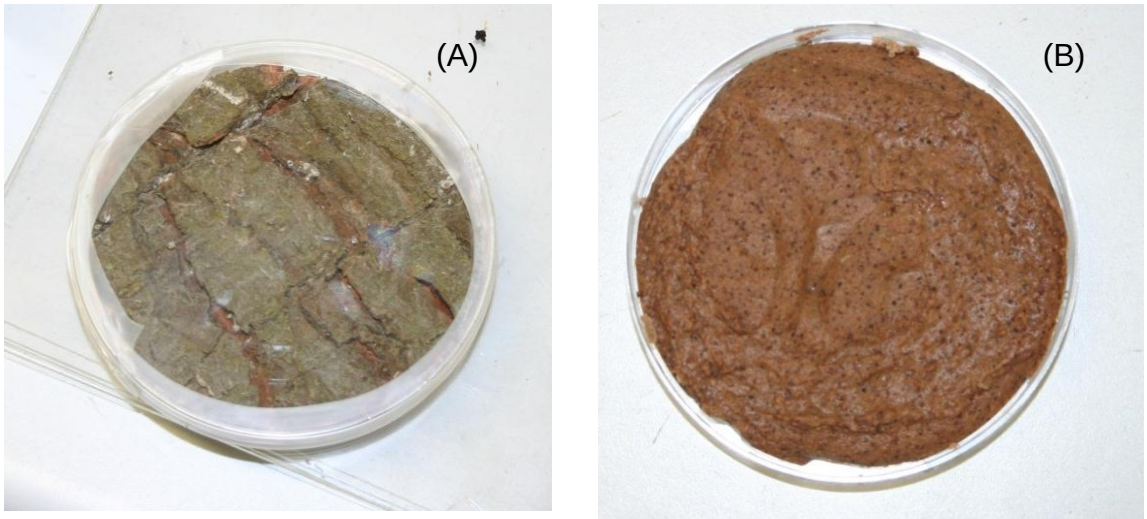

Figure 6. (A) *Hylurgus ligniperda* adults in a vented Petri dish with a bark disc for Experiments 1 and 2. (B) Petri dish containing diet on which *H. ligniperda* larvae, pupae and *A. ferus* larvae were kept for Experiment 2.

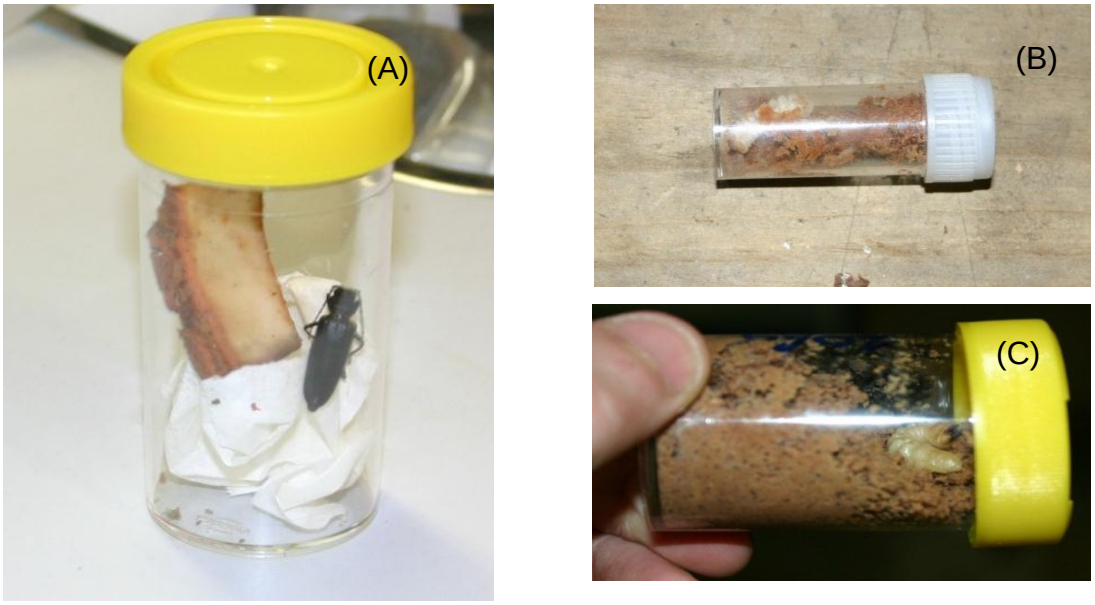

Figure 7. (A) *Arhopalus ferus* adult in 100ml container with moist paper towel and bark square, for Experiments 1 and 2. (B) *Arhopalus ferus* larvae in 10ml container for Experiment 2. (C) *Arhopalus ferus* larvae in 100ml container for Experiment 2.

3.5 Experiment 1 (Range finding)

A review of the International Database on Insect Disinfestations and Sterilizations (I.D.I.D.A.S) was undertaken to determine an appropriate initial dose range based on information from other coleopteran species. Information was ranked from lowest to highest dose and showed that 90% sterility was achieved for most species at <60 Gray (Gy) and that 95-100% sterility was achieved with doses from 25 - 160Gy (Tab. 1). Based on this review the dose range selected for this Experiment was set at 0 (control), 50, 100, 150, and 200Gy as it was thought to encompass both effective and non-effective doses and would capture the entire dose response curve.

Table 1. Summary of doses found in the IDIDAS literature (IAEA 2002) to achieve sterility in various coleopteran species.

Family	Genus/ species	Life stage	Sex	Dose (Gy)	Induced sterility (%)
Curculionidae	*Rhynchophorus ferrugineus*	Larvae	-	10	30
Curculionidae	*Rhynchophorus ferrugineus*	Pupae	-	15	55
Curculionidae	*Rhynchophorus ferrugineus*	Adult	Male	15	70-90
Anobiidae	*Lasioderma serricorne*	Egg	-	25	100
Anobiidae	*Stegobium paniceum*	Egg	-	25	99
Anobiidae	*Lasioderma serricorne*	Adult	-	30	Sterility
Anobiidae	*Stegobium paniceum*	Adult	-	30	Sterility
Curculionidae	*Pissodes strobi*	Adult	-	43.5	96-99
Anobiidae	*Lasioderma serricorne*	Larva	-	50	99
Bostrichidae	*Rhyzopertha dominica*	Adult	-	60	99
Anobiidae	*Anobium punctatum*	Adult	Male	69.6	-
Anobiidae	*Anobium punctatum*	Adult	Female	69.6	-
Anobiidae	*Lasioderma serricorne*	Egg	-	90	99.9
Anobiidae	*Stegobium paniceum*	Egg	-	90	100
Anobiidae	*Lasioderma serricorne*	Larva	-	120	99.9
Anobiidae	*Lasioderma serricorne*	Pupae	-	120	99
Anobiidae	*Stegobium paniceum*	Larva	-	120	100
Anobiidae	*Stegobium paniceum*	Pupae	-	120	100
Anobiidae	*Lasioderma serricorne*	Adult	-	125	100
Anobiidae	*Stegobium paniceum*	Adult	-	250	100
Bostrichidae	*Prostephanus truncatus*	Adult	-	60	100
Cerambycidae	*Anoplophora glabripennis*	Adult	Male	70-90	80
Cerambycidae	*Anoplophora glabripennis*	Adult	Female	70-90	100
Bostrichidae	*Prostephanus truncatus*	Adult	-	<150	96.7
Bostrichidae	*Rhyzopertha dominica*	Adult	-	<160	98-99

3.6 Experiment 2 (Replicated trial to pin-point sterilisation doses)

Irradiation was applied at doses of: 0 (control), 20, 40, 60, 80 and 100Gy. Three replicates of twenty individuals of each life stage (*A. ferus* adult, egg, and larvae, *H. ligniperda* egg, larvae, pupae, adult) were treated. A total of 60 individuals were subject to each dose, which meets the IPPC guidelines for testing irradiation as a phytosanitary measure (ISPM 18).

3.7 Post-irradiation monitoring

For the post irradiation monitoring sterility for adults, pupae and larvae it was defined as the failure of offspring develop from eggs into the larval life stage. For the egg life stage sterility was defined as the failure to develop from an egg to larvae.

Hylurgus ligniperda

Hylurgus ligniperda adults were reintroduced into bark discs in a vented Petri dish and were assessed at the end of a 37 day period to see if there any eggs, larvae or pupae present. Eggs of *H. ligniperda* placed on artificial diet were monitored every second day and presence of neonate larvae (hatchlings) were recorded. Emerging *H. ligniperda* larvae were reared on artificial diet and monitored, for survival until they pupated and developed into adults (50-64 days). Pupae were assessed after a 19 day period for the survival and emergence of adults. If pupae successfully emerged as adults they were placed on a bark disc in a ventilated Petri dish and were monitored for a 50 day period to observe the production of viable eggs.

Arhopalus ferus

Eggs were kept on moist paper towels in a Petri dish. Egg hatching was then monitored every second day and presence of neonate larvae recorded. Treated larvae were placed on artificial diet. Due to the long development time (212 days) survival to adulthood has not yet been assessed and is on-going. Treated adults were placed in opposite sex pairs in 100ml plastic vials with a moistened paper towel, and piece of bark and left to produce eggs. Vials were monitored every second day for egg production and the emergence of neonate larvae. All insect life stages were kept in a temperature-controlled chamber at 20 °C ± 1.5 after the irradiation.

3.8 Experiment 3 (Improved replicated *H. ligniperda* trial)

Experiment 2 did not capture the sterilisation dose for *H. ligniperda*. Therefore a third experiment was conducted with a higher dose range of 0, 100, 200, 300, 400, 500Gy.

Twenty individual eggs, larvae, adults and only five pupae were all contained within a single divided Petri-dish for this treatment (Fig. 8). Only five pupae were used for each treatment as the captive-reared larvae experienced high mortality on the artificial diet, reducing the number of individuals available at the time of treatment. Larvae and pupae were individually inserted into small holes in artificial diet. Eggs were placed on a piece of filter paper that sat on top of the pupae. Adults were sexed before treatment, so only ♀s were included in the experiment and these were placed under a thin piece of bark. Potentially 150 virgin females were required for pairing with. The virgin female *H. ligniperda* were lab reared by placing 320 larvae in individual diet tubes, assuming a male to female ratio of 50:50.

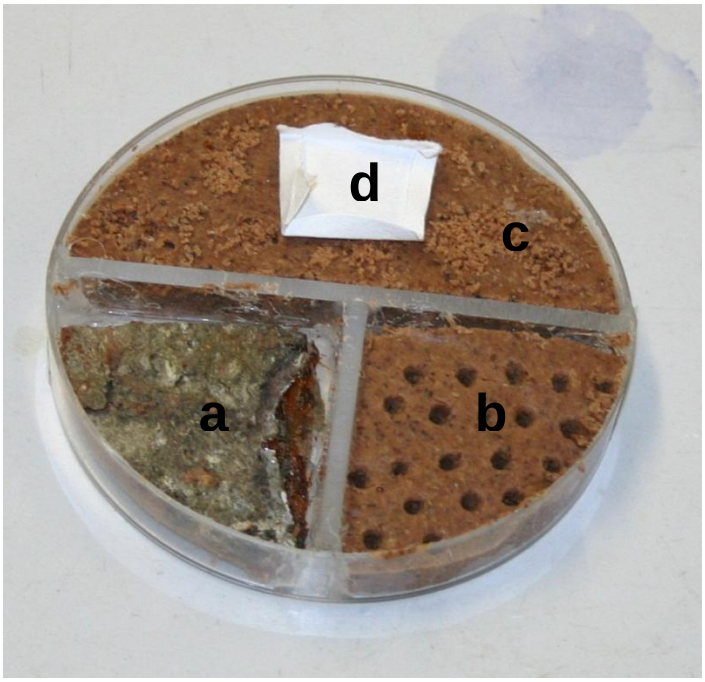

Figure 8. Segmented Petri dish containing (A) adult, (B) larvae and (C) pupae. (D) Eggs were then placed on top of the pupae on filter paper.

3.9 Post-Irradiation care & monitoring

The post monitoring for Experiment 3 is ongoing. Sterility for post irradiation monitoring of the egg life stage is defined as the failure of the egg to develop into the larvae life stage, while sterility for adults, pupae and larvae is defined as the

failure of offspring to develop from eggs into the larval life stage. As such irradiated larvae and pupae must be reared to adults and lay eggs before sterility can be assessed. If larvae do not hatch from these eggs this will indicated that the individuals are sterile.

The Adult ♀ *H. ligniperda* were paired with a single wild ♂ *H. ligniperda* and placed on a 5cm diameter bark disk in a vented Petri dish. Dishes were incubated at a constant 20°C for 25 days; then the bark discs were destructively sampled and assessed for survival, the presence of larvae and the presence of eggs.

Hylurgus ligniperda pupae were left in their artificial diet in a 20℃ growth chamber, until they reached adulthood. Emerging adults from the control were then individually transferred into a bark disc in a ventilated Petri dish with a partner of the opposite sex; either a virgin (lab-reared) ♀ *H. ligniperda* or a virgin ♂ *H. ligniperda*. The control paired adults were then incubated at a constant 20℃ for 25 days before bark discs were destructively sampled and assessed for survival, the presence of larvae and the presence of eggs. The treated pupae (100 – 500Gy) have not yet developed into adults. If they do develop, they will be paired for mating, as above, to assess sterility of any eggs they produce.

Hylurgus ligniperda larvae were transferred to individual diet tubes and incubated at 20℃. Emerging adults from the control treatment were sexed then placed individually on bark discs with opposite sex partners, as described above for the control *H. ligniperda* emerged pupae. The treated larvae have not yet developed, however if they do they will follow the same method as the control larvae that have emerged as adults.

Hylurgus ligniperda eggs were left on top of filter paper and stored in a 20℃ growth camber. The egg life stage was monitored every second day and emerged larvae were transferred onto a wedge under a bark disc. The replicate had a control failure, so the eggs that did hatch were not reared to adulthood to be assessed for sterility.

4. Results

4.1 Experiment 1 (Range finding)

4.1.1 *Arhopalus ferus* adults

Only 10% of control (0Gy) adult *A. ferus* failed to produce viable eggs (Fig. 9). Of the 30 individuals treated at each dose (50, 100, 150 and 200Gy) all were observed to be sterile (Fig. 9). The 100% sterility of adult *A. ferus* at 50Gy indicates that the effective dose to cause sterilisation is less than 50Gy. Because this was a range finding experiment there was no replication and thus it is not possible to model the effect of dose on survival to estimate the lethal dose (LD).

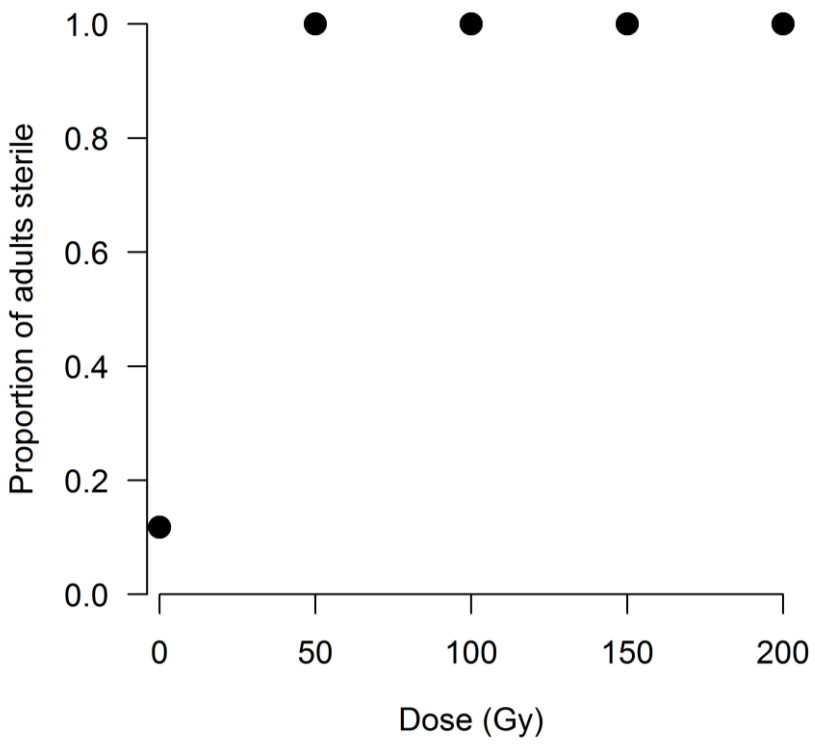

Figure 9. Proportion of all *A. ferus* adults rendered sterile following exposure to 0, 50, 100, 150 and 200Gy.

4.1.2 *Hylurgus ligniperda* adults

The adult life stage of *H. ligniperda* resulted in no useable data. This was due to a methodological error that did not allow the identification of individual sterility as all treated adults were placed together on a single bark disc.

4.2 Experiment 2 (Replicated trial to pin-point sterilisation doses)

After the range finding experiment a series of fully replicated experiments were conducted with 360 individuals per life stage for each of three independent replicates. Each life stage is discussed separately.

4.2.1 *Arhopalus ferus* adults

Although the sterility of the control individuals - was relatively high (compared to Experiment 1), it was much lower than the treated individuals, giving confidence that the treatment effects are 'real'. The dose of radiation applied to *A. ferus* adults significantly affected the level of sterility (GLM, Z=4.12, P<0.000) (Tab. 2). The fitted model showed that higher dose rates resulted in greater sterility and the GLM estimated a LD99 for *A. ferus* adults to be 30.2Gy with 95% confidence interval of 13.5Gy (Fig. 10). Given the relatively high mortality in two of the control treatments, It is suggest that further testing should be completed to achieve lower sterility in control individuals (Fig. 10).

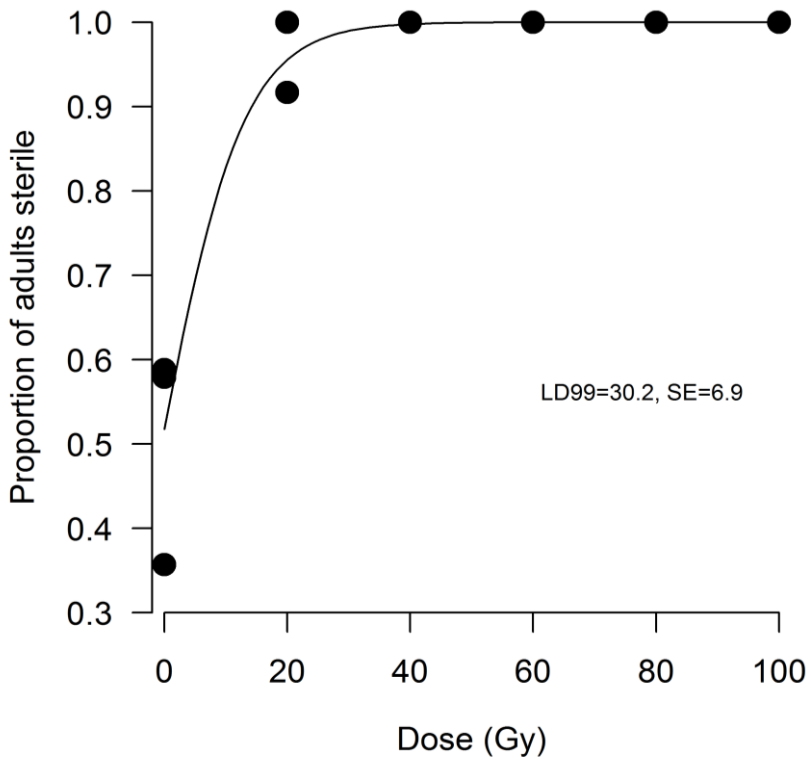

Figure 10. Proportion of all *A. ferus* adults found to be sterile following exposure to 0, 20, 40, 60, 80 and 100Gy with a generalised linear model (GLM) with a logarithmic curve fitted.

Table 2. GLM output for the analysis of the proportion of adult *A. ferus* found to be sterile following irradiation at 0, 20, 40, 60, 80 and 100Gy.

Coefficients:	Estimate	Standard Error	Z-value	P-value
Intercept	0.071	0.282	0.252	0.801
Dose	0.149	0.036	4.122	0.000

4.2.2 *Arhopalus ferus* larvae

The larval life stage of *A. ferus* has not yet been assessed as they take an average of 212 days to pupate. There has currently been no pupation and therefore no data is currently available for analysis. The survival of larvae in the control treatment was poor because some larvae attacked each other and/or escaped from their individual 100ml containers (see section 5.3.1).

4.2.3 *Arhopalus ferus* eggs

The sterility of the control individuals was relatively high; however it was much lower than the treated individuals for replicates 1 and 3. Replicate 2 had lower sterility in treated individuals than the controls (Fig. 11). Therefore the data is unsuitable and further testing should be completed to find the true effect of dose on *A. ferus* eggs. Although the model converged on a solution, the effect of radiation dose was only marginally significant, clearly influenced by the outcome of replicate 2 (GLM, $Z=1.932$, $P<0.053$) (Tab. 3). The GLM estimated a LD99 for *A. ferus* eggs to be 750Gy with 95% confidence interval of 776Gy (Fig. 11). Given the marginal significance of the model and the very large standard error of the LD99 estimate these results should be interpreted with caution.

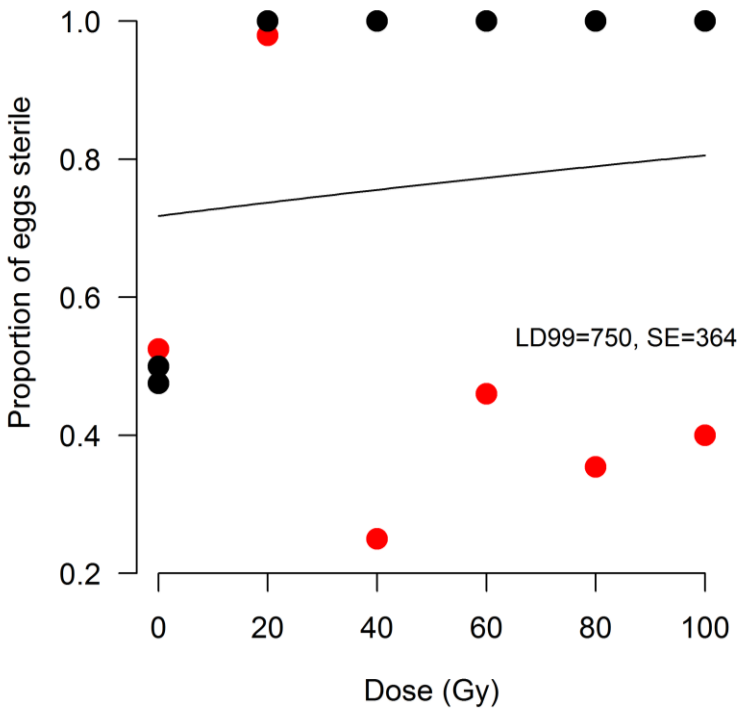

Figure 11. The proportion of all *A. ferus* eggs found to be sterile following exposure to 0, 20, 40, 60, 80 and 100Gy with a GLM with a logarithmic curve fitted. Replicate 1 and 3 shown in black and replicate 2 has been highlighted in red.

Table 3. GLM output for the analysis of the proportion of *A. ferus* eggs that were found to be sterile following irradiation at 0, 20, 40, 60, 80 and 100Gy.

Coefficients:	Estimate	Standard Error	Z-value	P-value
Intercept	0.933	0.148	6.306	0.000
Dose	0.005	0.003	1.932	0.053

4.2.4 *Hylurgus ligniperda* adults, pupal and larval life stages

Irradiation of adult, pupal and larval life stages of *H. ligniperda* resulted in no useable data. This was due to a methodological error that prevented identification of individual sterility (see section 5.3.1.).

4.2.5 *Hylurgus ligniperda* eggs

The survival of *H. ligniperda* eggs was quite variable between treatments; however, the proportion of control eggs that were sterile was significantly less than the proportion of treated individual's, giving confidence that the observed

treatment effects were ‘real’ (Fig12.). Treatments applied at 20 to 100 Gy were never 100% effective at rendering eggs sterile and therefore, higher dose should be tested. The effect of dose was significant, indicating that increasing dose resulted in more sterility (GLM, Z=5.197, P<0.000) (Tab. 4). The GLM estimated a LD99 for *H. ligniperda* eggs to be 289Gy with 95% confidence interval of 92Gy (Fig. 12).

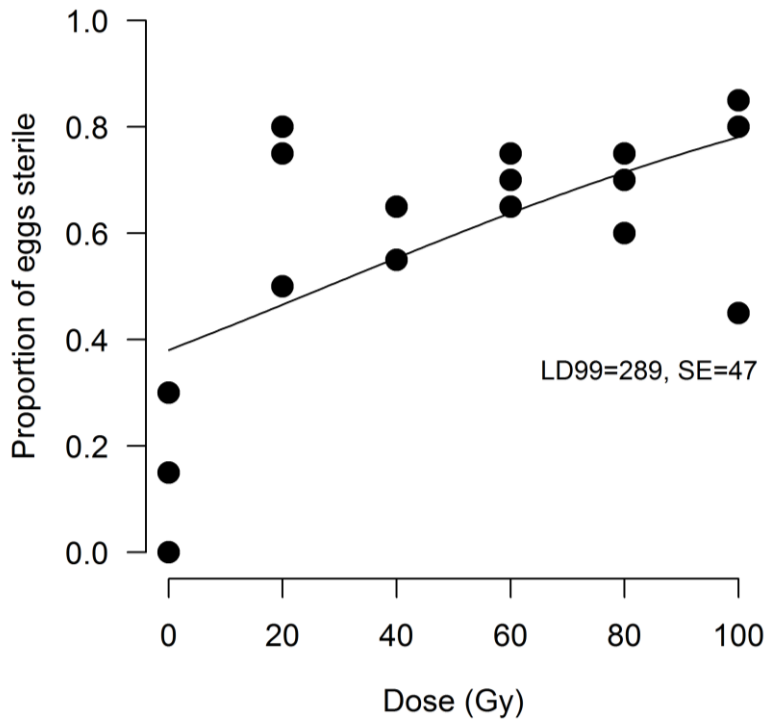

Figure 12. The proportion of *H. ligniperda* eggs found to be sterile following exposure to 0, 20, 40, 60, 80 and 100Gy with a GLM with a logarithmic curve fitted.

Table 4. GLM output for the analysis of the proportion of *H. ligniperda* eggs found to be sterile following irradiation at 0, 20, 40, 60, 80 and 100Gy.

Coefficients:	Estimate	Standard Error	Z-value	P-value
Intercept	-0.488	0.193	-2.532	0.011
Dose	0.018	0.003	5.197	0.000

4.3 Experiment 3

Experiment 3 is currently on going. At this point in time, no treated pupae or larvae at any dose have developed into adults; however control pupae and larvae have emerged as adults. This suggests that at 100Gy is enough to prevent development from the pupal and larval life stages to the adult life stage. However the radiation could have delayed there development, so 100Gy preventing development in the pupal and larval life stage could not be stated yet.

The egg life stage experienced a control failure, i.e. a large proportion of the control eggs did not hatch successfully. Therefore any effects or observations from the treated eggs were made invalid, as there were no controls to compare too.

The results from the adult life stage from replicate one have been included below and are looking positive, due to the improved experiment methodology.

4.3.1 *Hylurgus ligniperda* adults

Only 10% of adult *H. ligniperda* controls (0Gy) failed to produce viable eggs (Fig. 13). Of the 20 individuals treated at each dose (100, 200, 300, 400 and 500Gy) all except one at 100Gy were observed to be sterile (Fig. 13). The 100% sterility of adult *H. ligniperda* at 200Gy indicates that the effective dose for sterilisation is less than 200Gy. Only the first of three replicates in Experiment 3 have been completed at this point in time; thus it is not possible to model the effect of dose on survival to estimate the lethal dose (LD).

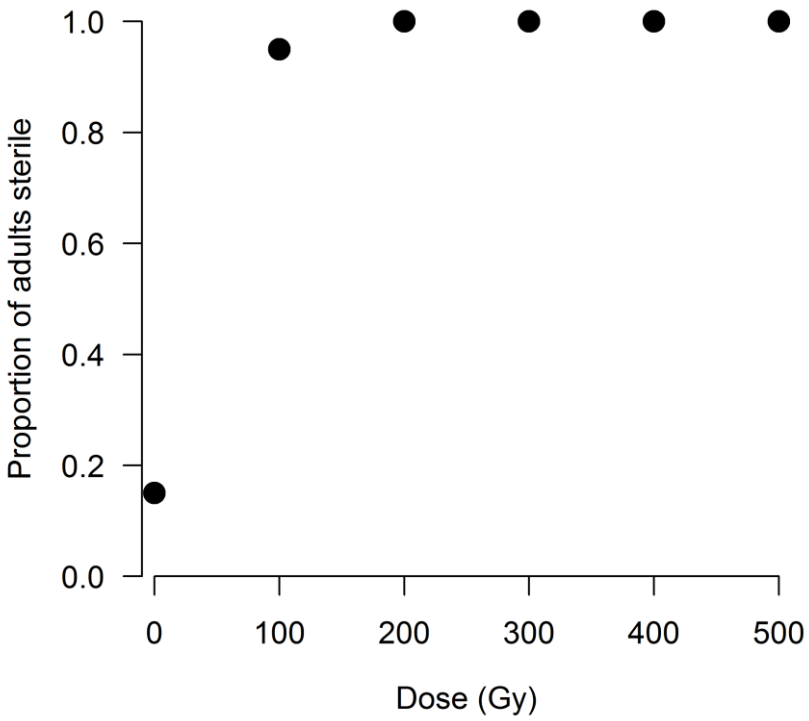

Figure 13. The proportion of treated *H. ligniperda* adults found to be sterile following exposure to 0, 100, 200, 300, 400 and 500Gy from replicate one only.

5. Discussion

Alternative phytosanitary treatments must be found to replace MeBr before a 2020 deadline beyond which it cannot be released to the atmosphere in New Zealand. Irradiation has been identified as a potential phytosanitary risk mitigation technique and its effectiveness to cause sterilisation of forest pests.

This study tested the null hypothesis that there is no association between dose and sterility of applicable life stages of *A. ferus* and *H. ligniperda.* The results from the GLM showed that there was a significant effect of radiation dose on sterility for *A. ferus* adults and *H. ligniperda* eggs; allowing the rejection of the null hypothesis for these two life stages. Therefore the main results of the study are that GLM analysis predicted a LD99 of 30.2Gy ±13.5Gy to induce sterilityof *A. ferus* adults and a LD99 of 289Gy ±92Gy to induce sterility of *H. ligniperda* eggs. The results for *H. ligniperda* adults, pupae, eggs and larvae of both species were inconclusive and are discussed below in detail in section 5.1.1.

5.1 Discussion and limitations of results from irradiated life stages

5.1.1 Adults

Arhopalus ferus adults

It was observed in Experiment 1, that *A. ferus* adults became sterile below 50Gy. This is below the range of doses (50-400Gy) found to render other coleopteran species sterile in ISPM 18, (IPPC, 2003). However the findings were consistent with IDIDAS (International Database on Insect Disinfestation & Sterilization) literature (IAEA 2002) that found sterility was achieved in various coleopteran species over a range of 25-160Gy. Experiment 1 results provided the rationale for the range of doses (0-100Gy) applied in the second experiment, to try and capture effective dose of sterilisation for *A. ferus* adults.

In Experiment 2, it was observed that *A. ferus* adults became sterile below 40Gy, with the GLM estimating an LD of 30.2Gy. These results were consistent with Experiment 1 in that sterility occurred below 50Gy, but there was a higher proportion of sterility in the control treatment (30-58%). Control sterility was, however, still considerably lower than the treated individuals, indicating that the treatment effects are 'real'. The LD99 of 30.2Gy determined in this study for *A. ferus* adults displays promise for a low dose treatment for sterilisation making the treatment more cost effective; although a full economic assessment would need to be conducted to assess this.

The LD99, is also less than the minimum standard range of 50-400Gy that has been stated in ISPM 18 for the sterilisation of Coleoptera (IPPC, 2003). This result could be used as a basis for negotiating a new phytosanitary treatment for sawn timber exports to Australia. Currently Australia recommends a treatment of 10,000Gy, but these preliminary results have indicated that the dose is significantly higher than necessary (Department of Agriculture Fisheries and Forestry, 2014). In order to justify this to our trading partners (Australia in this case), New Zealand would need to provide a higher level of significance such, as an LD99.99. The current data shows a LD99, which is a 1/100 chance of a pest surviving treatment, where as an LD99.99 is a 1/10,000 chance of a pest surviving treatment. Additional replication is required to achieve a higher level of confidence prior to producing a formal trade justification.

Hylurgus ligniperda adults

Sterility was not able to be assessed from data on *H. ligniperda* adults due to a methodological issue. All 20 adults treated at each dose were placed on a singular bark disc, meaning that the individual adult sterility could not be calculated in Experiments 1 and 2. The method has been revised for Experiment 3, as described below in section 5.2. The results from the first replicate of Experiment 3 were promising, indicating that <200Gy will cause sterility in *H. ligniperda* adults.

The same methodological issue was encountered for *H. ligniperda* pupae and larvae in Experiment 1. As a consequence there is no usable data for the pupal or larval life stage. This was resolved by following the new method developed as mentioned below in section 5.2.

5.1.2 Pupae

Arhopalus ferus pupae

Arhopalus ferus pupae were not treated due to this life stage being outside the dwell period of export radiata pine logs. The dwell period is the period from when the trees are felled to the point at which the logs are processed, which is less than 6 months for radiata pine logs. The larval stage takes 212 days on average to reach pupation; so the pupal life stage of *A. ferus* should not be present in radiata pine logs and therefore logs will not require treatment against this life stage.

5.1.3 Larvae

Arhopalus ferus larvae

Post monitoring is not yet completed for the *A. ferus* larval life stage. However, the *A. ferus* larvae suffered significant mortality during pre and post treatment care. Originally 20 larvae for each treatment-by-dose combination were kept in just two Petri dishes of artificial diet so that all individuals would be within the radiation zone during treatment. However the larvae are highly cannibalistic and in hindsight the larvae should have been transferred to individual habitats prior to treatment. Larvae were moved to separate 10ml vials of artificial diet post-treatment, however, a significant proportion escaped by eating through the vial lids. As all the individually labelled larval vials were kept in a single large container for each

replicate; it was therefore not possible to determine which dose escapees had been exposed to and the escaped individuals had to be discarded.

If this experiment is repeated, it is recommend the use of glass Petri dishes or glass containers, to prevent the larvae from escaping. I would also recommend that insects exposed to each dose are contained separately, then if larvae escape the dose and replicate could be easily identified.

5.1.4 Eggs

Arhopalus ferus eggs

The response of *A. ferus* eggs to radiation was inconsistent between replicates. In the control treatment there was minimal variation with all 3 replicates expressing approximately 50% sterility. Irradiated eggs in replicates 1 and 3 showed very consistent results with 100% of eggs becoming sterile after irradiation with 40Gy. However, in replicate 2 the proportion of sterility ranged from 25-45% at doses above 40Gy. This resulted in a high LD99 of 750Gy with a 95% confidence interval at 776Gy. If the results from replicate 2 were removed, the remaining *A. ferus* egg results would be consistent with the *A. ferus* adult results (sterility occurring between 20-40Gy, LD99= 30.2Gy). The reason for replicate 2 being inconsistent is unknown but is likely to be a biological issue relating to the egg age and development pre-irradiation.

Hylurgus ligniperda eggs

A sterilisation effect on *H. ligniperda* eggs was observed, however there was high variability between doses. Because of this variability an effective dose that would induce sterilisation was unable to be defined, as the predictive power of the model was poor. One of the most significant factors affecting the models prediction was the poor development of control individuals. In one replicate, 30% of eggs subject to 0Gy failed to develop. The control and the treated individuals also showed variation in the proportion of eggs found to be sterile between replicates, but the effect of the variable dose was still significant in the GLM analysis. The treated individuals at the highest doses did not show 100% egg sterility, indicating that the effective sterilisation dose was not captured. The GLM predicted a LD99 of 289Gy with 95% confidence intervals of 92Gy. These findings were incorporated into the

design of the third experiment such that the range of doses has been increased to 0-500Gy to capture the sterilisation dose.

5.2 Experiment 3- Methodological issues and proposed solutions

Experiment 3 has been designed to resolve methodological issues that prevented sterility being assessed for *H. ligniperda* in Experiment 1 and 2, as described above.

The method has been revised for Experiment 3 by only treating adult female *H. ligniperda* (sexed by testing for sounds emitted only by male individuals) and coupling them with a wild male on a bark disc in individual petri dishes. This enabled each to be assessed for sterility after 25 days. The same procedure will be used to assess sterility induced by irradiation of the pupae and larvae, once they have been reared to adulthood. The sex of pupae and larvae was not able to be determined before treatment and therefore the number of each sex present in the experiment will not be known until they reach adulthood. Females will be paired with lab reared virgin males. Males will be paired with a lab reared virgin female to ensure the female hadn't mated prior to being introduced to the treated males. This new method will allow the pupal and larval life stages to be assessed for sterility, after a 25 day period.

5.3 Limitations to implementation

5.3.1 Radiation source

This research has shown that irradiation may be a feasible phytosanitary treatment for the sterilisation of forestry pests because sterilisation of *A. ferus* can be achieved at a low dose (LD99 = 30.2Gy). Before progressing to an operational stage, however, a full techno-economic assessment relative to other options would have to be completed. A key factor of this assessment will be the source of irradiation, which could be a point of contention given New Zealand's nuclear free policy and public perceptions regarding the use of radioactive isotopes.

In these experiments a Cobalt-60 source was used as this was the only suitable radiation source in Christchurch and was installed in the 1960s. In the future, phytosanitary irradiation treatment facilities would be located in close proximity to ports and towns. Therefore acceptance by the public will need to be considered as part of the resource management process.

To implement irradiation as a phytosanitary treatment on an operational scale a combination of an E-beam and X-rays may be preferable radiation source. X-rays are energetically expensive but have the ability to penetrate further into materials allowing effectiveness at depth; whereas E-beams have a lower penetration depth but provide large doses quickly. Both systems are preferred over a cobalt source as they are powered by electricity and do not have a radioactive source. If the depth of penetration of New Zealand forestry pests into logs is demonstrated to be shallow during the time prior to export, then an E-beam alone may be the preferred option.

For forestry a customised installation set-up would be required for the irradiation of logs. A system using E-beams and x-rays for the irradiation of cylindrical objects has been proposed (Ivanov, Ovchinnikov, Svinin, Tolstun, & Bogart, 2000), which could be applied to radiata pine logs. This has potential, to be used at an industrial scale for phytosanitary risk mitigation (Ivanov, et al, 2000).

5.3.2 Next research stage

If Experiment 3 is successful then the most radiation-tolerant life stage of *H. ligniperda* will have been determined. Subsequently further testing (e.g. higher replication) could be concentrated on that life stage alone instead of all life stages, as all other life stages should reach sterility at the lower dose. However, the niche of each life stage will have a direct effect on its response to dose, because larvae that bury themselves into the sapwood of the tree will require a higher dose than those that stay in the phloem of the tree. This will need to be taken into account when determining the most radio-tolerant life stage.

This work contributes to what is known as phase 1 in ISPM 18 i.e. the testing of “naked” insects. This will then lead into phase 2, the testing of insects inside wood samples. Phase 1 establishes the most tolerant life stage, so phase 2 can concentrate on detailed testing of the most tolerant life stage. This significantly reduces the complexity of phase 2 testing and reduces the number of infested wood samples required and the number of insects that must be reared to conduct the testing, which is highly labour intensive. Finally phase 3 testing must occur in operational-scale trials. Operational trials would need to be conducted in an

irradiation facility to show that sterilisation can be achieved effectively and consistently on a production scale in compliance with the philosophy of ISPM 28.

5.3.3 Irradiation facility

There are currently no facilities in New Zealand that could irradiate whole logs and building such a facility for operational trials represents a significant financial risk to the developer. This is because our trading partners will not accept the treatment option until phase 3 operation trials confirm that the treatment is effective. Therefore a fully operational facility would have to be built with no guarantee of future business. However if research was completed through phase 1 and 2, with a high level engineering design of how the process would use the irradiation sources to mitigate the phytosanitary risk and identification of associated costs, it would significantly reduce the financial risk to a developer, making it a better investment opportunity.

6. Conclusion

The study has shown that 30.2Gy ± 13.5Gy is sufficient to sterilise *A. ferus* adults, which is significantly less than the 10,000Gy what is required for Australian sawn timber exports. It was also shown that 289Gy ±92Gy will induce sterility *H. ligniperda* eggs. Therefore this study achieved sterilisation doses to a LD99 significance level for 2 out of 7 applicable life stages for *A. ferus* and *H. ligniperda*. Results for the *A. ferus* egg life stage were found to be insignificant which was caused by the effect of replicate 2 being inconsistent with the other two replicates. However if the replicate 2 data was excluded, it shows consistencies with the *A.ferus* adult data. Results for the *A. ferus* larval life stage and the adult, pupal and larval *H. ligniperda* life stages were inconclusive due to methodological issues. However these issues were detected and rectified for the third experiment for *H. ligniperda* life stages. After one replication, the third experiment has shown promising results which suggest sterility may be induced in the adult stage between 100 and 200Gy.

Irradiation as an alternative phytosanitary measure for MeBr is promising as the foundational work has been done for agricultural products; it just needs to be adopted with appropriate changes for use in forestry. Due to the uncertain public acceptability of irradiation as a large-scale phytosanitary treatment and the business risks associated with building an irradiation facility to confirm that sterilisation can be operationally achievable it will be it hard to establish irradiation as a treatment in the near future. However, this research is the first step in assessing the viability of irradiation as a phytosanitary risk mitigation technique. To reduce the business risk further testing should be completed on the New Zealand forestry pests, the irradiation source, the engineering facility design and the associated costs. This would reduce the associated financial risk and make it a more viable phytosanitary risk mitigation technique.

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